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Differences in the Responses of Two Mudskippers to Terrestrial Exposure

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ABSTRACT—The concentration of NH_4^+ in the plasma and muscles of *Periophthalmodon schlosseri* and *Boleophthalmus boddaerti* increased significantly after exposure to terrestrial conditions for 24 hr. Such increases in NH_4^+ concentrations in *B. boddaerti* were not accompanied by any significant increase in urea concentrations. However, significant increases in urea concentrations occurred in the plasma, liver and muscle of *P. schlosseri* kept away from water. Results obtained indicate that the liver may be a major organ involved in urea production in this mudskipper. In addition, there were significant increases in the concentrations of total free amino acids in the plasma, liver and muscle of *P. schlosseri,* but not in those of *B. boddaerti,* after 24 hr of terrestrial exposure. Terrestrial exposure also affected the aminating and deaminating activities of glutamate dehydrogenase from the liver of *P. schlosseri*, leading to a significant increase in the aminating/deaminating ratio. It is concluded that *P. schlosseri*, having developed a greater affinity to land than *B. boddaerti,* has also acquired a greater capacity to detoxify ammonia.

INTRODUCTION

Mudskippers are gobioid teleosts usually found in mangrove swamps in the estuaries of rivers. They are amphibious and spend a substantial part of their lives out of water. In Singapore, *Boleophthalmus boddaerti* and *Periophthalmodon schlosseri* inhabit mud flats which are periodically inundated by the tide. The former makes burrows on the lower regions of the intertidal zone while the latter burrows on higher ground. At low tide, both are found on the mudflats. At high tide, *B. boddaerti* stays in its water-filled burrows and resurfaces only when the tide ebbs while *P. schlosseri* often swims with its snout and eyes above water along the water's edge.

Recent studies by low *et al.* [17, 18] reveal that the gills of *P. schlosseri* are better adapted to a terrestrial than an aquatic environment. It has relatively fewer and shorter gill filaments, and its gills exhibit intrafilamentary secondary lamellar fusions which reduce coalescence of the respiratory surfaces upon terrestrial exposure. However, such fusions would render branchial gaseous exchange in water *via* the counter-current mechanism inefficient. In contrast, the gills of *B. boddaerti* are better adapted for aquatic respiration as they exhibit relatively longer gill filaments, and most of their secondary lamellae are aligned to the respiratory water current.

In general, the nature of the major nitrogenous end-product of a species is correlated with that species's environment: aquatic species are ammonotelic, whereas terrestrial species are either ureotelic or purinotelic [3, 21]. Gordon *et al.* [9] first demonstrated that the Madagascan mudskipper, *Periophthalmus sobrinus*, might have a potential for the transition to ureotelism while it was out of water. Similar results were obtained for the Chinese mudskipper, *Periophthalmus cantonensis* [10]. However, Morii *et al.* [22, 23] and Morii [21] showed that NH_4^+ was mainly accumulated, and its conversion to urea was hardly performed, in the bodies of *P. cantonensis* and *Boleophthalmus pecti-*

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nirostris during the period out of water. Iwata *et al.* [15] obtained results, similar to those of Morii [21] and Morii *et al.* [22, 23], on *P. cantonensis* in a separate study.

To date, whether mudskippers have a potential for the transition towards ureotelism during a terrestrial excursion is still disputable. This is mainly due to the lack of knowledge on their differences in terrestrial affinities. It would appear that *P. schlosseri* is a more important candidate to be examined in this regard because its gills show the highest degree of adaptation, amongst the mudskippers, to respire terrestrially. Since no such information on this mudskipper is available, the present study was undertaken to elucidate the mechanisms by which ammonia was detoxified in *P. schlosseri* when exposed to a terrestrial environment. Experiments were also performed on the relatively more aquatic species, *B. boddaerti,* for comparison. During the course of the study, it was observed that, different from *B. boddaerti, P. schlosseri* accumulated in its tissues not only urea and NH_4^+, but also significantly higher concentrations of total free amino acids (TFAA) after 24 hr of terrestrial exposure. Therefore, the possible involvement of glutamate dehydrogenase (GDH) in ammonia detoxification in the latter mudskipper was also examined.

MATERIALS AND METHODS

Collection and maintenance of mudskippers

P. schlosseri and *B. boddaerti* were collected along the estuarine canal at Pasir Ris, Singapore. They were maintained in 50% (15‰ salinity) seawater (SW) at 25°C in the laboratory and the SW was changed daily. The aquaria were tilted slightly, so that the fish were free to be in or out of water. No attempt was made to separate the sexes. *P. schlosseri* and *B. boddaerti* were fed small guppies and a manufactured product (Goldfish and Staple Flake, Everyday Co., Singapore), respectively.

Exposure of mudskippers to experimental conditions

Fish were exposed to terrestrial conditions at 25°C for 24 hr in aquaria lined with one layer of cotton wool and two layers of Whatman no. 1 filter paper steeped with 50% SW at the bottom. After 24 hr, fish were anaesthetized for 10 min in an atmosphere saturated with diethylether.

Fish submerged in aerated 50% SW at 25°C for 24 hr were used as controls for comparison. After 24 hr in the SW, fish were anaesthetized by the introduction of 3-aminobenzoic acid ethyl ester (MS222) at a final concentration of 0.005%.

Sample preparation for the analyses of NH_4^+, urea and free amino acids (FAA)

Anaesthetized fish were killed immediately by pithing. The lateral muscle and the liver were quickly excised. No attempt was made to separate red and white muscles. The excised tissues and organs were immediately freeze-clamped in liquid N_2 with precooled tongs [6]. The whole procedure was completed within 30 s. Frozen samples were kept at −80°C until analysis.

The frozen samples was weighed, ground to a powder under liquid N_2 and placed in either 5 vol (w/v) (for urea and NH_4^+ analyses) or 15 vol (w/v) (for FAA analyses) of ice-cold 6% trichloroacetic acid (TCA). The sample was homogenized thrice using an Ultra-Turrax homogenizer (Janke and Kunkel Gmbh & Co., Germany) at maximum speed for 20 sec each with 10 sec off intervals. The sample was then centrifuged at 10,000×*g* at 4°C for 10 min using a Kokusan H251 refrigerated centrifuge (Kokusan Enshinki Co., Japan). The supernatant fluid obtained was analyzed for NH_4^+, urea and FAA.

A separate group of fish exposed to similar conditions was used for the collection of blood samples. The caudal peduncle of the anaesthetized fish was severed and blood exuding from the caudal artery was collected in heparinized capillary tubes. The tubes were centrifuged at 4,000×*g* at 4°C for 10 min to obtain the plasma. The collected plasma was deproteinized in 2 vol (v/v) of ice-cold 6% TCA and centrifuged at 10,000×*g* at 4°C for 15 min. The resulting supernatant fluid was kept at −80°C until analysis.

Determinations of NH_4^+, urea and FAA

For the analysis of NH_4^+, the pH of the depro-

teinized sample was adjusted to 5.5–6.0 with 5 M $KHCO_3$. The NH_4^+ content was determined according to the method of Kun and Kearney [16]. The reaction medium, in a total of 2.7 ml, contained 100 mM Tris-HCl (pH 8.0), 10 mM α-ketoglutarate ($\propto$KG), 0.19 mM NADH, 24.3 IU GDH (Sigma Chemical Co., MO) and 0.2 ml sample. Freshly prepared NH_4Cl solution was used as the standard for comparison.

For the determination of urea, the pH of the sample was neutralized with 5 M K_2CO_3. To 0.2 ml of this neutralized sample, analysis was performed using a Sigma Urea Assay Kit Procedure 535 (Sigma Chemical Co., MO). To another 0.2 ml of the same sample, similar analysis was performed after incubating for 15 min at 30°C with 0.2 ml of 20 mM imidazole buffer (pH 7.0) containing 2 IU urease (Sigma Chemical Co, MO). The difference in absorbance obtained from samples with and without urease treatment was used for the estimation of the urea concentration in the sample. Urea obtained from Sigma Chemical Co. (USA) was used as a standard for comparison.

For the analysis of FAA, the sample was adjusted to pH 2.2 with 4 M LiOH and diluted appropriately with 0.2 M lithium citrate buffer (pH 2.2). FAA were analyzed using a Shimadzu LC-6A Amino Acid Analysis System with a Shim-pack ISC-07/S1504 Li type column. Since taurine was found to be present in much greater concentrations than other FAA in the various tissues and organs of these two mudskippers (except in the muscle of *B. boddaerti*) and since terrestrial exposure had no significant effect on its concentrations, the authors decided not to take it into account in the calculation for the concentration of TFAA to better reflect the overall changes in the concentrations of other FAA.

Results were expressed as μM/g for muscles and livers, and μM/ml for plasma.

Preparation of samples for enzymatic analyses

Samples for GDH assays were prepared according to Chew and Ip [4] with some modifications. The excised muscle was homogenized in 5 vol (w/v) of an ice-cold buffer, which contained 300 mM sucrose, 0.1 mM EDTA and 3 mM Tris-HCl (pH 7.4), using an Ultra-Turrax homogenizer at minimum speed for 10 sec. The liver sample was homogenized three strokes in 10 vol (w/v) of the same buffer using a teflon-glass homogenizer. The homogenized sample was centrifuged at $600\times g$ for 15 min at 4°C in a Beckman J2-21 M/E refrigerated centrifuge (Beckman Instruments, USA) to remove any unbroken cells and nuclei. The supernatant fluid obtained was further centrifuged for 15 min at $10{,}000\times g$ to obtain the mitochondria. The mitochondria were washed twice by resuspension and resedimentation in the same buffer and sonicated before assaying of GDH activity.

Enzyme assays

Enzyme assays were performed by monitoring changes of absorbance at 25°C using a Shimadzu UV-160A spectrophotometer.

Activity of GDH in the aminating direction was determined according to the method of Iwata *et al.* [15] with some modifications. The reaction mixture in a total volume of 2.8 ml contained 200 mM triethanolamine-HCl (pH 7.8), 0.1 mM NADH, 0.12 mM ADP, 10 mM αKG and 250 mM ammonium acetate. A control assay was conducted without αKG. The oxidation of NADH was monitored at 340 nm. Specific activities were expressed as μmol NADH oxidized/mg protein per min.

Activity of GDH in the deaminating direction was assayed using a modified colorimetric method of Beutler and Michal [2]. The absorbance change was monitored at 492 nm. The reaction medium, in a total volume of 1.3 ml, comprised 200 mM glycine-NaOH (pH 8.8), 0.4 mM NAD, 0.12 mM ADP, 0.8 mM iodonitrotetrazolium chloride, 0.17 IU/ml diaphorase (Sigma Chemical Co., USA) and 100 mM glutamate. The specific activity was expressed as μM formazan formed/mg protein per min.

Protein content of the sample was determined according to the method of Bradford [1]. Bovine gamma globulin (Sigma Chemical Co., USA) dissolved in 25% glycerol was used as the standard for comparison.

Determination of LT_{50} for mudskippers exposed to various concentrations of NH_4Cl

Because of the great difference in ammonia tolerance between the two mudskippers, different

concentrations of NH_4Cl were used to obtain the respective LT_{50} values. Ten *B. boddaerti* (20–30 g) were exposed individually at 25°C to 3.7 liter of aerated SW containing 20 or 50 mM of NH_4Cl (Merck Chemical Co., Germany). Similarly, 10 *P. schosseri* (90–120 g) were exposed to 150 mM NH_4Cl. The time of mortality for each group was recorded. The LT_{50} values were determined graphically.

Statistical analyses

Results were presented as mean ± SE. Student's *t*-test was used to compare differences between means. Differences with $P<0.05$ were regarded as statistically significant.

RESULTS

The concentrations of NH_4^+ in the plasma and muscles of *P. schlosseri* and *B. boddaerti* increased significantly after exposure to terrestrial conditions for 24 hr (Table 1). Under both the submerged and terrestrial conditions, the NH_4^+ concentrations in the liver of *P. schlosseri* were approximately 4 times higher than those of *B. boddaerti*. The increases in NH_4^+ concentrations in the plasma and muscle of *B. boddaerti* were not accompanied by any significant increase in urea concentrations. However, significant increases in urea concentrations occurred in the plasma, liver and muscle of *P. schlosseri* kept away from water (Table 1). The increase in the concentration of urea, without an accompanying increase in that of NH_4^+, in the liver of *P. schlosseri* exposed terrestrially led to a significant increase in the urea/NH_4^+ ratio in this organ.

The liver of *P. schlosseri* contained a significantly greater concentration of TFAA than that of *B. boddaerti* (Table 2). However, the TFAA concentration in the muscle of the latter was significantly higher than that of the former (Table 3). This was due to the presence of approximately 20 times more glycine in the muscle of *B. boddaerti* compared to that of *P. schlosseri*. The taurine concentrations in the liver (Table 2) and muscle (Table 3) of *P. schlosseri* were significantly higher than those of *B. boddaerti*.

After exposure to terrestrial conditions for 24 hr, there were significant increases in the concentrations of TFAA in the liver (Table 2), muscle (Table 3) and plasma (Table 4) of *P. schlosseri*, but not in those of *B. boddaerti*. In the latter

Table 1. Concentrations (μM/ml plasma and μM/g liver or muscle) of urea and ammonia and their ratios (urea/ammonia) in the plasma, liver and muscle of *B. boddaerti* and *P. schlosseri* fully submerged in 50% SW or exposed to terrestrial condition for 24 hr[1]

Fish	Tissues	Conditions	Urea	Ammonia	Urea / Ammonia
B. boddaerti	Plasma	Submerged	1.34±0.09	0.44±0.03	2.90±0.32
		Terrestrial	1.61±0.13	0.72±0.05*	2.09±0.27
	Liver	Submerged	0.55±0.07	0.64±0.07	0.91±0.17
		Terrestrial	0.59±0.15	0.88±0.09	0.66±0.13
	Muscle	Submerged	0.81±0.05	0.88±0.07	0.93±0.12
		Terrestrial	0.79±0.12	1.46±0.20*	0.59±0.11
P. schlosseri	Plasma	Submerged	1.14±0.16	0.54±0.06	2.17±0.29
		Terrestrial	2.34±0.32*	0.92±0.05*	2.19±0.33
	Liver	Submerged	0.61±0.13	2.54±0.21	0.23±0.05
		Terrestrial	1.66±0.21*	3.46±0.54	0.50±0.08*
	Muscle	Submerged	0.89±0.14	0.96±0.13	1.05±0.31
		Terrestrial	1.47±0.13*	1.64±0.09*	0.90±0.08

[1] Results represent means ± SE of three of five determinations on separate preparation from different animals.
* Significantly different from the corresponding value of the submerged fish.

TABLE 2. Concentrations (μM/g) of various free amino acids (FAA) and total FAA (TFAA) in the livers of *B. boddaerti* and *P. schlosseri* fully submerged in 50% SW or exposed to terrestrial condition for 24 hr[1]

FAA	*B. boddaerti*		*P. schlosseri*	
	Submerged	Terrestrial	Submerged	Terrestrial
Ala	0.22 ±0.02	0.15 ±0.03	1.17 ±0.12	4.13±0.74*
Arg	0.020±0.002	0.020±0.002	0.18 ±0.03	0.19±0.04
Asp	0.26 ±0.04	0.24 ±0.03	0.27 ±0.03	0.43±0.04*
Glu	1.96 ±0.32	3.10 ±0.47	4.20 ±0.29	5.39±0.31*
Gly	1.09 ±0.08	0.61 ±0.15*	1.04 ±0.12	0.68±0.09*
His	0.17 ±0.02	0.16 ±0.03	0.26 ±0.02	0.33±0.04
Ile	0.040±0.006	0.039±0.005	0.080±0.007	0.16±0.01*
Leu	0.130±0.020	0.094±0.014	0.21 ±0.02	0.37±0.02*
Lys	0.15 ±0.01	0.14 ±0.03	0.39 ±0.02	0.98±0.30
Phe	0.038±0.007	0.052±0.003	0.21 ±0.02	0.22±0.04
Pro	0.24 ±0.05	0.28 ±0.05	0.36 ±0.03	0.59±0.08*
Ser	0.17 ±0.01	0.13 ±0.02	0.10 ±0.01	0.18±0.02*
Thr	0.040±0.004	0.042±0.007	0.94 ±0.10	0.39±0.05*
Tyr	0.17 ±0.05	0.13 ±0.009	0.09 ±0.02	0.16±0.02
Val	0.18 ±0.04	0.22 ±0.04	0.24 ±0.02	0.60±0.11*
TFAA	5.13 ±1.06	5.59 ±0.80	10.3 ±0.8	15.5±1.4*
Tau	7.54 ±0.34	7.23 ±1.47	22.2 ±3.4	20.2±2.3

[1] Results represent means±SE of six to seven determinations on separate preparations from different animals.

* Significantly different from that of the corresponding value of the submerged fish.

TABLE 3. Concentrations (μM/g) of various free amino acids (FAA) and total FAA (TFAA) in the muscles of *B. boddaerti* and *P. schlosseri* fully submerged in 50% SW or exposed to terrestrial condition for 24 hr[1]

FAA	*B. boddaerti*		*P. schlosseri*	
	Submerged	Terrestrial	Submerged	Terrestrial
Ala	1.95±0.09	2.94±0.26*	1.30±0.12	2.99±0.32*
Arg	0.49±0.05	0.28±0.01*	0.18±0.02	0.33±0.03*
Asp	0.33±0.05	0.12±0.02*	0.13±0.01	0.11±0.02
Glu	0.42±0.06	0.31±0.02	0.23±0.05	0.22±0.03
Gly	22.6±1.0	23.0±1.3	1.07±0.13	1.30±0.16
His	0.71±0.13	1.03±0.03*	0.25±0.02	0.32±0.03
Ile	0.22±0.02	0.20±0.01	0.12±0.01	0.35±0.03*
Leu	0.41±0.04	0.36±0.01	0.27±0.02	0.66±0.05*
Lys	2.17±0.07	2.08±0.11	1.16±0.14	1.63±0.14
Phe	0.07±0.01	0.11±0.03	0.17±0.03	0.24±0.02
Pro	0.19±0.01	0.27±0.02*	0.10±0.02	0.24±0.01*
Ser	0.90±0.07	0.92±0.02	0.23±0.01	0.44±0.05*
Thr	0.32±0.06	0.54±0.04*	0.31±0.02	0.42±0.04*
Tyr	0.25±0.01	0.17±0.02*	0.10±0.03	0.18±0.02
Val	0.28±0.03	0.26±0.02	0.21±0.02	0.51±0.04*
TFAA	31.6±0.8	32.2±1.3	5.99±0.33	9.93±0.78*
Tau	7.40±0.49	9.05±1.93	19.4±1.9	19.1±2.9

[1] Results represent means±SE of five to six determinations on separate preparations from different animals.

* Significantly different from that of the corresponding value of the submerged fish.

Table 4. Concentrations (μM/ml) of various free amino acids (FAA) and total FAA (TFAA) in the plasma of *B. boddaerti* and *P. schlosseri* fully submerged in 50% SW or exposed to terrestrial condition for 24 hr[1]

FAA	*B. boddaerti*		*P. schlosseri*	
	Submerged	Terrestrial	Submerged	Terrestrial
Ala	0.043 ±0.002	0.071 ±0.004*	0.140 ±0.018	0.242 ±0.019*
Arg	0.023 ±0.002	0.025 ±0.007	0.044 ±0.019	0.056 ±0.003
Asp	0.0070±0.0012	0.0067±0.0006	0.0041±0.0001	0.0034±0.0002
Glu	0.013 ±0.002	0.014 ±0.002	0.014 ±0.001	0.012 ±0.003
Gly	0.248 ±0.013	0.260 ±0.020	0.076 ±0.008	0.078 ±0.009
His	0.019 ±0.001	0.021 ±0.005	0.022 ±0.004	0.027 ±0.003
Ile	0.051 ±0.005	0.047 ±0.001	0.063 ±0.004	0.156 ±0.006*
Leu	0.115 ±0.012	0.103 ±0.005	0.109 ±0.009	0.256 ±0.015*
Lys	0.020 ±0.003	0.056 ±0.007	0.057 ±0.019	0.139 ±0.031*
Phe	0.023 ±0.002	0.022 ±0.004	0.034 ±0.004	0.049 ±0.007*
Pro	0.0090±0.0006	0.0110±0.0010	0.018 ±0.001	0.036 ±0.008*
Ser	0.016 ±0.001	0.017 ±0.001	0.024 ±0.002	0.025 ±0.003
Thr	0.020 ±0.001	0.025 ±0.004	0.072 ±0.008	0.069 ±0.009
Tyr	0.022 ±0.003	0.024 ±0.001	0.021 ±0.002	0.036 ±0.005*
Val	0.073 ±0.007	0.066 ±0.003	0.121 ±0.007	0.195 ±0.026*
TFAA	0.721 ±0.030	0.762 ±0.041	0.886 ±0.075	1.359 ±0.088*
Tau	8.26 ±1.21	9.14 ±0.98	10.0 ±2.5	12.2 ±1.4

[1] Results represent means±SE of five to six determination on separate preparations from different animals.
* Significantly different from that of the corresponding value of the submerged fish.

Table 5. Specific enzyme activities of glutamate dehydrogenase in the amination (μM NADH oxidized/mg protein per min) and deamination (μM formazan formed/mg protein per min) directions, and their ratios (amination/deamination) from the livers and muscles of *B. boddaerti* and *P. schlosseri* fully submerged in 50% SW or exposed to terrestrial condition for 24 hr[1]

Fish	Tissues	Conditions	Amination	Deamination	Amination / Deamination
B. boddaerti	Liver	Submerged	0.18 ±0.03	0.0072±0.0008	21.8±2.2
		Terrestrial	0.25 ±0.03	0.0068±0.0006	28.2±2.7
	Muscle	Submerged	0.028±0.001	0.0022±0.0001	12.8±0.8
		Terrestrial	0.025±0.001	0.0022±0.0001	11.7±0.7
P. schlosseri	Liver	Submerged	1.62 ±0.21	0.0372±0.0060	58.1±12.0
		Terrestrial	0.85 ±0.06*	0.0088±0.0014*	116 ±21*
	Muscle	Submerged	0.13 ±0.03	0.0075±0.0015	17.7±0.6
		Terrestrial	0.20 ±0.03	0.0098±0.0018	19.3±2.0

[1] Results represent means±SE of five to six determinations on separate preparations from different animals.
* Significantly different from the corresponding value of the submerged fish.

mudskipper, only the concentration of alanine in the plasma was significantly increased after terrestrial exposure. However, similar exposure increased the concentrations of alanine, isoleucine, leucine, lysine, phenylalanine, proline, tyrosine and valine in the plasma of *P. schlosseri* (Table 4). There were significant decreases in glycine concentrations in the livers of both *B. boddaerti* and *P. schlosseri* exposed to terrestrial conditions (Table 2). On the other hand, the concentrations of alanine, aspartate, glutamate, isoleucine, leucine, proline, serine and valine increased significantly in the liver of only *P. schlosseri* after 24 hr of terrestrial exposure (Table 2). Terrestrial exposure significantly decreased (arginine, aspartate and tyrosine) and increased (alanine, histidine, proline and threonine) the concentrations of several FAA in the muscle of *B. boddaerti* (Table 3). In comparison, only significant increases in the concentrations of 8 FAA (alanine, arginine, isoleucine, leucine, proline, serine, threonine and valine) were observed in the muscle of *P. schlosseri* exposed to terrestrial conditions (Table 3). Terrestrial exposure had no significant effect on the concentrations of taurine in the liver (Table 2), muscle (Table 3) and plasma (Table 4) of both mudskippers.

The specific activities of GDH, in the aminating and deaminating directions, in the liver and muscle of *P. schlosseri* were significantly higher than those of *B. boddaerti* (Table 5). Terrestrial exposure had no significant effect on the aminating and deaminating activities of the GDH from the liver and muscle of *B. boddaerti.* In contrast, similar exposure decreased the aminating and deaminating activities of the GDH from the liver of *P. schlosseri*, but the amination/deamination ratio was significantly increased (Table 5).

The LT_{50} of *P. schlosseri* for 150 mM NH_4Cl was 13 hr. For *B. boddaerti*, the LT_{50} for 20 and 50 mM NH_4Cl were 75 and 36 min, respectively.

DISCUSSION

Since branchial NH_4^+ excretion would be inefficient when no external water current is available to irrigate the gills during terrestrial exposure, the plasma and muscles of *P. schlosseri* and *B. boddaerti* exposed terrestrially for 24 hr accumulated significantly greater quantities of NH_4^+ compared to those of the respective fish submerged in water. Similar to *P. cantonensis* and *B. pectinirostris* [21–23], terrestrial exposure had no significant effect on the urea concentrations in the plasma, liver and muscle of *B. boddaerti.* Hence, the accumulated NH_4^+ does not seem to be channelled into urea production in *B. boddaerti.* In contrast, *P. schlosseri* accumulated significantly greater concentrations of urea in the plasma, liver and muscle after being kept away from water for 24 hr. Thus, a conversion of NH_4^+ into urea might have occurred in this mudskipper during terrestrial exposure. The authors speculate that the liver may be the major organ involved in urea production in *P. schlosseri,* because this organ exhibited the greatest increase (2.5 fold) in urea content but without any significant accumulation of NH_4^+ after 24 hr of terrestrial exposure, leading to a significantly greater urea/NH_4^+ ratio compared to that of the submerged fish.

The pathway involved in the increased urea synthesis in *P. schlosseri* during terrestrial exposure is not elucidated in this study. Some teleosts can synthesize urea through purine catabolism in their liver [5, 8, 20]. Gregory [11] detected urate oxidase, allantoicase and allantoinase, which are involved in converting uric acid through allantoin and allantoic acid to glyoxylate and urea, in the mudskippers, *Periophthalmus expeditionium* and *Periophthalmus gracilis.* Glyoxylate could lead to the formation of glycine through transamination reactions. Since the glycine level in the liver of *P. schlosseri* exposed to 24 hr of terrestrial conditions was significantly lower than that of the submerged ones, it can be deduced that the increased urea production in this fish during terrestrial exposure may not be due to the degradation of uric acid. The ornithine-urea cycle is an alternative pathway for urea synthesis in some other teleosts [13, 20, 24]. However, Gregory [11] was able to detect only two (arginase and ornithine carbamoyltransferase) out of five enzymes of the ornithine-urea cycle in the liver extracts from the mudskippers, *P. expeditionium, P. gracilis* and *Scartelaos histophorus.* Since no information concerning this aspect is available for *P. schlosseri,* we are currently investigating if all the enzymes of this cycle are present in

this mudskipper.

Iwata *et al.* [15] reported that the muscle of *P. cantonensis* accumulated FAA, especially the non-essential ones, after terrestrial exposure. However, in *P. schlosseri,* accumulations of some essential amino acids, e.g. the branched-chain FAA, also occurred. Hence, there might be an increase in the mobilization of protein as carbon and energy sources in *P. schlosseri* exposed to terrestrial conditions. Such a shift in metabolic pathways may be related to the high terrestrial affinity of this mudskipper. It may be metabolically more active under the terrestrial condition as its gills are better adapted to respire on land than in water [17, 18]. Indeed, it is the only fish known to exhibit bradycardia when submerged in water ([7]; Ip, unpublished results).

A closer examination of the FAA profiles reveals that the major FAA accumulated in the tissues of *P. schlosseri* exposed to terrestrial conditions was alanine. The percentages of increases in TFAA which could be accounted for by the respective increases in alanine contents in the liver, muscle and plasma of *P. schlosseri* exposed terrestrially were approximately 57%, 22% and 56%, respectively. Such a phenomenon was not observed in *P. cantonensis* [15]. It has been demonstrated that most of the amino acids released by proteolysis in the white muscle of the spawning salmon during migration are collected and transported to other tissues as alanine [19]. Therefore, it is possible that a similar strategy is adopted by *P. schlosseri* during terrestrial exposure. According to our current understanding of pathways of amino acid metabolism, most of the free amino acid pool can be converted to alanine [12]. The overall quantitative energetics of these processes in white muscles appear to be quite favorable; the net conversion of glutamate to alanine would yield 10 mol ATP per mole alanine formed, and this value can be even higher for proline and for arginine conversion to alanine [12]. Since this favourable ATP yield from partial amino acid catabolism is not accompanied by a net release of NH_4^+, it would be advantageous to *P. schlosseri* which is confronted with the problem of NH_4^+ excretion when it moves away from water. For such a system to work, the NH_4^+ released from the partial catabolism of various FAA must be incorporated into glutamate, which can then undergo transamination to transfer the amino group to pyruvate, leading to the formation of alanine. It would appear that a portion of the NH_4^+ produced by *P. schlosseri* during the 24 hr of terrestrial exposure could be shuttled to the liver, and be converted into glutamate through amination of αKG. Although the specific activities (approaching V_{max}) of GDH obtained do not necessarily reflect the enzyme activities *in vivo*, the amination/deamination ratio in the liver of this mudskipper significantly increased after 24 hr of terrestrial exposure, which correlates well with the significant increase in glutamate content in this organ.

Contrary to *P. chrysospilos* [15] and *P. schlosseri, B. boddaerti* did not exhibit any increase in the TFAA and branched-chain FAA contents in its tissues when exposed terrestrially. Since there was no change in the glutamate level and the specific activities of GDH, in the aminating and deaminating directions, in the liver of *B. boddaerti* after 24 hr of terrestrial exposure, the formation of alanine in the muscle of this fish might not involve the amination of αKG through GDH in the liver as in *P. schlosseri.* Instead, the formation of alanine from certain FAA in the muscle of *B. boddaerti* during terrestrial exposure might involve transamination reactions within this tissue.

That the increases in concentrations of urea and FAA in the tissues of *P. schlosseri* exposed to terrestrial conditions indeed signal a greater capacity to detoxify ammonia, and not merely an increase in the mobilization of protein and amino acids, is reflected by the high tolerance of this mudskipper to NH_4^+ added to the ambient SW. Although it is generally accepted that fish are especially intolerant of dissolved ammonia [3], *P. schlosseri* could survive for at least 10 days in 100 mM NH_4Cl (Ip, unpublished results), and its LT_{50} for 150 mM NH_4Cl was 13 hr. To the best of the authors' knowledge, *P. schlosseri* exhibits one of the highest tolerance to NH_4^+ amongst teleosts, and even amongst mudskippers [14].

Hence, it can be concluded that, even though both are mudskippers, *P. schlosseri* and *B. boddaerti* have developed different degrees of adapta-

tion to survive in a terrestrial environment. The former mudskipper has acquired a greater capacity to detoxify ammonia than the latter. Thus, NH_4^+ excretion would be less of a problem to *P. schlosseri* than *B. bodaerti* during a terrestrial excursion.

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